Corning® BioCoat™ Growth Factor Reduced

Matrigel® Invasion Chamber

Catalog No. 354483

Lot No.

Guidelines for Use

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
CLSTechServ@Corning.com www.corning.com/lifesciences

CORNING

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

TABLE OF CONTENTS

I. INTENDED USE 2

II. SUMMARY 2

III. QUALITY CONTROL 3

IV. PRECAUTIONS 3

V. STABILITY 3

VI. INVASION OF METASTATIC CELLS 3

A. Materials Provided 3

B. Materials Required But Not Provided 4

C. Procedure For Use 4

D. Typical Results 8

VIII. REFERENCES 9

IX. ORDERING INFORMATION 9

X. TECHNICAL SERVICES 9

NOTE: Process changes have been made to improve the performance and quality of Corning® BioCoat™ Growth Factor Reduced Matrigel® Invasion Chambers. Please review the following Sections for new information regarding product usage.

PRECAUTIONS (b) (page 3): New storage conditions
PROCEDURE FOR USE (page 4): Appearance of the Matrigel Basement Membrane Matrix
PROCEDURE FOR USE, Section 1.0 Rehydration (page 4) New rehydration times
PROCEDURE FOR USE, Section Invasion Studies (page 5): New suggested cell seeding densities

Discovery Labware, Inc., Two Oak Park, Bedford, MA 01730, Tel: 1.978.442.2200 (U.S.)
CLSTechServ@Corning.com www.corning.com/lifesciences

CORNING

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

LOT NUMBER:

I. INTENDED USE

The Corning® BioCoat™ Growth Factor Reduced (GFR) Matrigel® Invasion Chamber is useful to study cell invasion of malignant and normal cells. The standard Corning BioCoat Matrigel Invasion Chamber has been used for the assessment of the metastatic potential of tumor cells[1], inhibition of metastasis by extracellular matrix components[2] or antineoplastic drugs (taxol)[3] altered expression of cell surface proteins in metastatic cells,[4] and invasion of normal cells, such as embryonic stem cells,[5] cytotrophoblasts,[6] and fibroblasts.[7]

Invasion studies have been successfully performed on a variety of tumor cells (cell lines and primary tumors) including melanomas, glioblastomas, astrocytomas, osteosarcomas, fibrosarcomas, and adenocarcinomas of the lung, prostate, breast, ovary, and kidney.

Corning Matrigel Matrix, a solubilized tissue basement membrane preparation, contains laminin, collagen type IV, heparan sulfate proteoglycan, entactin, growth factors, and other components. By employing a modified method developed by Taub, et al[8] a more defined preparation of Corning Matrigel Matrix is obtained where the levels of endogenous growth factors, excluding TGF-b are greatly reduced. This modified form, Growth Factor Reduced (GFR) Matrigel is used due to its reduced levels of EGF, PDGF, and IGF-1, which have been shown to influence cell behavior (Vukicevic, et al[9]).

Since the levels of growth factors are significantly reduced in the GFR Matrigel Matrix, these Invasion Chambers can be used to study the mechanisms of invasion and to identify factors that interfere with this process in a complex, yet relatively well defined, *in vitro* environment.

This product is **FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES**.

II. SUMMARY

Corning BioCoat GFR Matrigel Invasion Chambers provide the conditions to assess the invasive properties of cells *in vitro*. The Corning BioCoat GFR Matrigel Invasion Chamber consists of two Falcon® TC Companion Plates, each containing twelve Falcon Cell Culture Inserts with 8 micron pore size PET membrane. Each membrane has a thin layer of GFR Matrigel Basement Membrane Matrix that serves as a reconstituted basement membrane *in vitro*. The layer occludes the pores of the membrane, blocking non-invasive cells from migrating through the membrane. However, cells that migrate in response to the presence of a chemoattractant beneath the insert are able to detach from and invade through the GFR Matrigel matrix and the 8 micron membrane pores. The membrane may be processed for light and electron microscopy and can be easily removed after staining. The GFR Matrigel Invasion Chamber is a convenient, ready-to-use system to study the process and mechanisms of cell invasion *in vitro*.

LOT NUMBER:

III. QUALITY CONTROL

Corning® GFR Matrigel® Basement Membrane Matrix Invasion Chambers is evaluated for invasivity using HT1080 cells - an invasive human fibrosarcoma cell line - and 3T3 cells - a mouse fibroblast cell line of low invasivity. Approximately 750 microliters of 5% fetal bovine serum in tissue culture medium is added to the plate well as a chemoattractant and 500 microliters of cell suspension at approximately 5 x 10^4 cells/ml is added to the chamber (2.5 x 10^4 cells/24-well chamber). Chambers are incubated at 37°C for 24 hours. Noninvasive cells are removed from the upper surface of the membrane with a cotton swab before staining and microscopically counting invasive cells on the underside of the membrane. Invasivity of HT1080 cells has been found to be in the range of 4 to 10-fold higher than for 3T3 cells.

IV. PRECAUTIONS

a) Storage: Materials should be stored at -20°C in the original packaging.

b) All procedures should be performed under aseptic conditions.

California Proposition 65 Notice

WARNING:	This product contains a chemical known to the state of California to cause cancer.
Component:	**Chloroform**

V. STORAGE

The Corning BioCoat™ GFR Matrigel Invasion Chambers are stable when stored at -20°C.

VI. INVASION OF METASTATIC CELLS

NOTE: The following procedure has been optimized using HT-1080 human fibrosarcoma cells. Results may vary depending upon the cells used and the specific conditions under which the procedure is performed, especially those of medium, incubation time, cell seed density, and chemoattractant. Individual researchers should optimize conditions for their system.

A. Materials Provided

LOT NUMBER:

- Corning BioCoat GFR Matrigel Invasion Chambers (354483) provided as 12 inserts in each of two 24-well Falcon® TC Companion Tissue Culture Plates.

B. Materials Required But Not Provided

- Serum free culture medium such as DMEM containing 0.1% bovine serum albumin
- Corning® BioCoat™ Control Inserts, 24-well (Catalog No. 354578)
- Chemoattractant such as 5% fetal calf serum in tissue culture media
- Falcon® Companion Plates, 24-well (Catalog No. 3504)
- Humidified tissue culture incubator, 37°C, 5% CO_2 atmosphere
- Diff-Quick™ staining kit (Baxter Catalog No. B4132-1)
- Laminar flow tissue culture hood
- Scalpel (# 11 blade recommended)
- Microscope with (optional) camera
- Microscope slides and coverslips
- Cotton swab
- Sterile forceps
- Immersion oil

C. Procedure For Use

The appearance of the Corning Matrigel® Matrix coating on the Corning BioCoat Growth Factor Reduced Matrigel Invasion Chamber has changed. This is a result of process improvements which have enhanced the performance and quality of the product.

1.0 Rehydration

NOTE: a) See Table 1 for volume to be used for the Corning BioCoat GFR Matrigel Invasion Chambers.

b) Remove the foil bag containing the Corning BioCoat GFR Matrigel Invasion Chambers from -20°C and allow bag to come to room temperature.

c) Do not return Invasion Chambers to -20°C after warming product to room temperature.

LOT NUMBER:

Table 1. Solution Volumes
for Corning BioCoat GFR Matrigel Invasion Chambers

Procedure	Volume
Rehydration	0.5 ml
Plate Wells	0.75 ml
Added Cells	0.5 ml
Stains	0.5 ml

1.1 Add 0.5 ml warm (37°C) culture medium to the interior of the inserts. Allow to rehydrate for **2 hours** in a humidified tissue culture incubator at 37°C, 5% CO_2 atmosphere.

1.2 After rehydration, carefully remove medium without disturbing the layer of Corning® BioCoat™ GFR Matrigel® Matrix on the membrane.

2.0 Invasion Studies

2.1 Rehydrate the GFR Matrigel inserts to be used as directed above. Prepare an equal number of Corning BioCoat Control Inserts (no GFR Matrigel coating) by using sterile forceps to transfer them to empty wells of the Falcon® TC Companion Plate.

2.2 Prepare cell suspensions in culture medium containing 0.1% BSA at **5 x 10^4 cells/ml**.

2.3 Add chemoattractant to the wells of the Falcon TC Companion Plate.

2.4 Use sterile forceps to transfer the GFR Matrigel and control inserts to the wells containing the chemoattractant. Be sure that no air bubbles are trapped beneath the membranes. This can be avoided by tipping the insert at a slight angle as it is lowered into the liquid.

2.5 Immediately add 0.5 ml of cell suspension (**2.5 x 10^4 cells**) to the inside of the inserts.

LOT NUMBER:

2.6 Incubate the Corning BioCoat GFR Matrigel Invasion Chambers and Controls for 18 - 24 hours in a humidified tissue culture incubator at 37°C, 5% CO_2 atmosphere.

3.0 Measurement of Cell Invasion

3.1 Removal of non-invading cells

NOTE: After incubation, the non-invading cells are removed from the upper surface of the membrane by "scrubbing." As the attachment of the membrane to the insert housing is quite firm, the membrane will not be dislodged during scrubbing. Scrubbing is very efficient in removing GFR Matrigel and non-invading cells from the upper membrane surface. **Scrubbing must be accomplished quickly to avoid drying of the cells adhering to the bottom surface of the membrane**.

a.) Insert a cotton tipped swab into the GFR Matrigel insert and apply gentle but firm pressure while moving the tip over the membrane surface.

b.) Repeat the scrubbing with a second swab moistened with medium.

3.2 Staining of cells

NOTE: Depending upon the proportion of cells that invade, cell counting may be facilitated by counting Diff-Quick™ stained cells from photographs of the membrane or by directly counting the stained cells at the microscope.

Procedure for Using Diff-Quick Stain

The cells on the lower surface of the membrane may be stained with Diff-Quick stain. The Diff-Quick kit contains a fixative and two stain solutions. The appearance is similar to that obtained by Wright-Giesma staining. The cell nucleus stains purple and the cytoplasm stains pink. Suitable alternative staining procedures include fixation followed by hematoxylin and eosin or crystal violet. The membranes need not be removed from the insert housing for staining.

a.) Add each Diff-Quick solution to a row of a Falcon® TC Companion Plate. Add distilled water to two beakers.

b.) Sequentially transfer the inserts through each stain solution and the two beakers of water. Allow approximately 30 seconds in each solution.

c.) Allow the inserts to air dry for minimum of 60 minutes.

3.3 Counting of invading cells

LOT NUMBER:

NOTE: Cell counting is facilitated by photographing the membrane through the microscope. Direct counting of the cells at the microscope is also acceptable.

a.) Remove the membrane from the insert housing by inverting the insert and inserting the tip of a sharp scalpel blade through the membrane at the edge adjacent to the housing wall. Rotate the insert housing against the stationary blade and the membrane will be released in much the same manner as the lid is cut from a tin can. Do not fully release the membrane from the housing but leave a very small point of attachment.

b.) Use forceps to peel the membrane from the remaining point of attachment and place it bottom-side-down on a microscope slide on which a small drop of immersion oil has been placed. Place a second very small drop of immersion oil on top of the membrane.

c.) Place a second slide or coverslip on top of the membrane and apply gentle pressure to expel any air bubbles.

d.) Observe and/or photograph the invading cells under the microscope at approximately 100X magnification. Count the cells in the central field of triplicate membranes.

3.4 Data Reduction

NOTE: Data is expressed as the percent invasion through the Corning® BioCoat™ GFR Matrigel® Matrix and membrane relative to the migration through the Control membrane. The "Invasion Index" is also expressed as the ratio of the percent invasion of a test cell over the percent invasion of a control cell.

a.) Determine Percent Invasion:

$$\% \text{ Invasion} = \frac{\text{Mean \# of cells invading through GFR Matrigel insert membrane}}{\text{Mean \# of cells migrating through control insert membrane}} \times 100$$

b.) Determine Invasion Index:

$$\text{Invasion Index} = \frac{\% \text{ Invasion Test Cell}}{\% \text{ Invasion Control Cell}}$$

LOT NUMBER:

D. Typical Results

The following results are typical of those obtained when the Corning Biocoat GFR Matrigel Invasion Chambers are used as described to assess the invasion of HT-1080 fibrosarcoma test cells and NIH 3T3 control cells in an 18 - 24 hour assay. **They are provided for reference only**. Results will vary with different cell types, chemoattractants, and incubation time.

Table 2

	HT-1080 (test cells)			NIH 3T3 (control cells)		
# cells invading (triplicate)	95	119	103	10	4	7
Mean	105.6			7.3		
# cells invading (control inserts)	388	391	343	231	264	308
Mean	374			267		
% Invasion	105.6/374 x 400 = 28.24%			2.33/98 x 100 = 2.8%		
Invasion Index	28.2÷2.8 = 10.1					

LOT NUMBER:

VIII. REFERENCES

1. Albini, A. et al., Cancer Reserach, 47:3239 (1987).
2. Kobayshi H., et al., Cancer Research, 52:3610 (1992).
3. Melchiori, A. et al., Cancer Research, 52:2352 (1992).
4. Stearns, M. and Want, M., Cancer Research, 52:3776 (1992).
5. Alexander, C.M. and Werb, A., Cell Biology, 118:727 (1992)
6. Librach, C.L. et al, Cell Biol., 113:437 (1991).
7. Chu, Y-W et al., Proc. Natl. Acad. Sci USA, 90:4261 (1993).

IX. ORDERING INFORMATION

To place an order in the U.S., contact Customer Service at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSCustServ@corning.com.

Outside the U.S., contact your local distributor or visit www.corning.com/lifesciences to locate your nearest Corning office.

X. TECHNICAL SUPPORT

Email CLSTechServ@Corning.com for regional Technical and Scientific Support.

LOT NUMBER: